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ISOLATION OF A LIVING BACTERIAL CULTURE OF BRUCELLA FROM A QUASI-STERILE SEMILIQUID ANTIBRUCELLOSIS FORMOL VACCINE

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As shown in the published investigations by G. M. Bosh'yan (cf G. M. Bosh'yan, On the Nature of Viruses and Microbes, 1949), formalin does not kill bacteria and viruses when it is applied in the customary concentrations of 0.1-1% in the process of preparing vaccines. According to Bosh'yan, formalin under these conditions simply combines with the viruses and microbes, transforming them into a biologically less active form which is less pathogenic or totally nonpathogenic. The transformation into the less active form insures survival of the particular species of microbe or virus. When appropriate conditions have been created, the viruses and microbes may again be transformed into the initial, biologically active, form.

On the basis of Bosh'yan's discoveries, we carried out an investigation on the isolation of a living Brucella culture from a "sterile" semiliquid antibrucellosis formol vaccine. For this investigation we used a 100-ml bottle containing supposedly sterile antibrucellosis formol vaccine (series No 31, control No 31, produced on 16 April 1949 by the Kaluga Biologicals Plant). A superficial examination of the bottle showed that nothing was wrong with the label or seal. The bottle was filled with a uniformly turbid, whitish grey liquid, which did not contain any extraneous admixtures or any lumps which did not break up. In smears made from the vaccine, small gram-negative polymorphous coccus bacteria and rods of the Brucella type were found after staining by the method of Gram.

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Using Bosh'yan's method, we isolated in a number of experiments a living bacterial culture from the sterile vaccine supplied to us and investigated this culture thoroughly as far as its morphological, propagative, and antigenic properties are concerned. In the first generations, the isolated culture presented the appearance of gram-positive granularity of various sizes and shapes. However, in the course of subsequent work which involved the exertion of influence in a definite direction on the above-mentioned culture, it became identical with laboratory Brucella strains from the standpoint of morphological, propagative, and biological properties.

Notwithstanding the fact that the investigation was conducted by setting up a number of parallel experiments which were different in character, identical strains of Brucella were obtained in all cases. These strains were indicated by us with No 13, 13-P;, 13-Pk, 15_1, 15_2, etc. The culture we isolated consists of small gram-negative coccus bacteria, which are occasionally elongated, so that they approach the shape of rods. The bacteria are disposed individually and occasionally in pairs. They do not form spores. Morphologically, the isolated culture cannot be distinguished from Brucella. It grows well under aerobic conditions on the media which are commonly used for the cultivation of Brucella, i.e., MPPA (meat-peptone-liver agar) and MPPB (broth).

After a Petri dish filled with MPPA has been seeded, the culture grows in the form of small, almost transparent dew-drop colonies. After aging, these colonies acquire a grayish tinge. When grown on an inclined MPPA medium, the culture first forms a tender, semitransparent, bluish-gray film. With advancing growth of the culture, the film coarsens and becomes more opaque. In MPPB, the culture forms an homogenous turbidity and a precipitate. The strains we isolated grow well on different media, but do not form either acid or gas in media containing dextrose, mannitol, saccharose, or maltose. They also do not modify the litmus-milk medium or curdle milk. In other words, these strains behave like Brucella. When grown on MPPA, the bacteria usually evolve hydrogen sulfide and, at the expiration of 48 hours, cause blackening of a strip of filter paper soaked in a solution of lead acetate.

All strains also grow well on solid and semiliquid liver media containing dyestuffs: with fuchsin in a dilution of 1:25,000, thionin in a dilution of 1:50,000, methyl violet in a dilution of 1:100,000, and pyronin in a dilution of 1:200,000 they behave like Brucella melitensis. Tables 1 and 2 cite data which characterize the growth of strains No 13, 13-Pp, 13-Pk, 15_1, and 15_2 on the above-mentioned culture media.

The living culture of bacteria we isolated from the vaccine was introduced into guinea pigs. It induced formation of agglutinins in the blood of these animals that were active against the typical brucellosis antigen. Table 3 lists data on the investigation of the blood of guinea pigs. It can be seen from these tables that the blood serum of guinea pigs No 5977 and No 5932, which were infected with the bacterial culture isolated from the antibrucellosis vaccine, gave a pronounced agglutination reaction with the typical brucellosis antigen in dilutions down to 1:200. The agglutinins appeared in the blood of these animals after the same period of time as in the case of vaccination with the identical dosis of the formol vaccine under investigation (see Table 3 for the results obtained with guinea pig No 9063). The titer of agglutinins was even higher than that which resulted after vaccination with formol vaccine.

In conclusion, it must be noted that strains we isolated are agglutinated at definite stages by specific sera obtained from diseased animals infected with brucellosis (positive brucellosis serum of cattle). The strains we isolated are thus identified as Brucella.

Conclusions

1. By using G. M. Bosh'yan's method, we isolated from the "sterile" semiliquid antibrucellosis formol vaccine, series No 31, a living bacterial culture which is identical with Brucella melitensis.

2. Formalin in the concentrations used for the preparation of sterile antibrucellosis vaccine does not kill, but only inactivates the causative factor of the infection in question. Under the action of formalin, the mode of existence and properties of the causative factor are changed.

Under definite conditions the modified, but still living, bacterial culture of the "sterile" vaccine may acquire the form and properties which are typical for the initial strain.

/Appended tables follow/

Table 1. Biochemical Activity of Various Strains of Bacteria Isolated From Antibrucellosis Vaccine, Series No 31

	Medium							
Strain	Dextrose	Lactose	Mannitol	Saccharose	Maltose	Litmus-milk	Milk	Formation of H_2S
13	+	+	+	+	+	+	+	+
13 - Pp	+	+	+	+	+	+	+	+
13 - Pk	+	+	+	+	+	+	+	+
15_1	+	+	+	+	+	+	+	+
15_2	+	+	+	+	+	+	+	+
Museum /standard/ strain of Brucella melitensis	+	+	+	+	+	+	+	+

Table 2. Growth of Strains Isolated From Antibrucellosis Vaccine, Series No 31, on Liver Media Containing Dyestuffs

	Concentration of Dyestuff in Medium			
Strain	Fuchsin 1:25,000	Thionin 1:500,000	Methyl violet 1:100,000	Pyronin 1:200,000
13	+	+	+	+
13 - Pp	+	+	+	+
13 - Pk	+	+	+	+
15_1	+	+	+	+
15_2	+	+	+	+
Museum /standard/ strain of Brucella melitensis	+	+	+	+

+ Indicates growth not accompanied by acid formation, evolution of gas, and modifications of milk.

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Table 3. Titer of Agglutinins in the Blood of Infected Guinea Pigs After Various Periods Have Elapsed Since Initial Infection

No of Guinea Pig	Agent Used	Titer of Agglutinins After Various Periods			
		Before Infection	After 10 Days	After 20 Days	After 27-31 Days
5977	Culture isolated from [redacted]	-	1:200 + + +	1:50 + +	-
5982	Culture isolated from vaccine	-	1:200 + + +	1:50 + +	-
9063	Vaccine under investigation (Series No 31)	-	1:50 + + + +	1:100 + +	1:100 + +
9070	Vaccine under investigation (Series No 31)	-	-	-	-

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